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Award Number: DAMD17-01-1-0304

TITLE: Reduction of Radiation- or Chemotherapy-Induced Toxicity by Specific Expression of Anti-Apoptotic Molecules in Normal Cells

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REPORT DATE: June 2004

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2004	Final (1 Jun 2001 - 31 May 2004)

4. TITLE AND SUBTITLE
Reduction of Radiation- or Chemotherapy-Induced Toxicity by Specific Expression of Anti-Apoptotic Molecules in Normal Cells

5. FUNDING NUMBERS
DAMD17-01-1-0304

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Adjuvant radiation and chemotherapy confer a survival benefit in breast cancer , but both treatments can damage normal tissues in ways that can adversely affect quality of life (e.g., by skin desquamation, mucositis, pulmonary fibrosis, cardiomyopathy, peripheral neuropathy). These effects on normal tissues are generally due to apoptosis (programmed death) of normal cells. We hypothesize that ectopic overexpression of the anti-apoptotic molecule Bcl-2 will inhibit the radiation-induced apoptosis of normal cells and thus reduce the toxicity of these treatments. We found that overexpressing Bcl-2 in murine fibroblast NIH3T3 cells resulted in resistance to radiation. Heterogeneous plasmid that expresses Bcl-2 cDNA in front of a minimal promoter regulated by multiple wild-type p53 DNA-binding sites protects specifically cells with wild-type p53—but not p53-mutated or p53-deleted cancer cells from genotoxic damage (e.g., radiation) by upregulated expression of p53 and Bcl-2. Progress is described the results of specific aim 2 and 3 that allowed to development of preclinical animal model.

14. SUBJECT TERMS
Apoptosis, gene therapy, chemotherapy, radiation therapy, toxicity, quality of life

15. NUMBER OF PAGES
9

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Introduction

The toxic effects of radiation and chemotherapy on normal tissues is a significant problem for patients with breast cancer because those effects worsen quality of life (QOL) and hinder the ability to tolerate these conventional therapies at effective levels. Our long-range goals are to reduce the toxicity associated with radiation therapy or chemotherapy and to improve the QOL of patients with breast cancer. One mechanism by which radiation and chemotherapy produce toxic effects is through inducing apoptosis of normal cells in normal tissues. We hypothesize that ectopic overexpression of anti-apoptotic molecules will inhibit the radiation- or chemotherapy-induced apoptosis of normal cells and thereby reduce the toxicity of these treatment modalities. We have shown that overexpression of the anti-apoptotic gene Bcl-2 can protect normal breast epithelial cells from apoptosis *in vitro,* but whether this effect will reduce the toxicity associated with radiation or chemotherapy in patients is unknown. Cells that contain wild-type (wt) p53 typically react to the genotoxic stress of radiation or chemotherapy by upregulating the expression of p53, which binds to specific DNA sequences and activates specific genes, some of which activate apoptosis. To prevent expression of anti-apoptotic genes by cancer cells, we exploited the fact that many types of cancer cells lack or have mutated forms of p53 and are developing a construct in which expression of anti-apoptotic genes is driven a minimal promoter under the control of the wt p53 binding sequence. This strategy is expected to limit the expression of anti-apoptotic genes to normal cells, thus reducing the risk that breast cancer cells become chemo- or radioresistant because of inappropriate overexpression of anti-apoptotic molecules. We expect that cells under genotoxic stress (by being exposed to chemotherapy or radiation) will express higher levels of anti-apoptotic molecules owing to upregulation of p53. Finally, we will use LPD cationic liposomes to create a novel, nonviral gene delivery system for systemic delivery of these anti-apoptotic molecules to normal organs such as lung, liver, kidney, and spleen. This proposal is innovative in that it seeks to prevent or reduce the toxic side effects of conventional therapies (i.e., radiation and chemotherapy) by inhibiting the fundamental biological process of "apoptosis" that they induce in a wt p53–specific manner. The results obtained may lead to the discovery of effective ways to protect normal tissues from radiation or chemotherapy without reducing the efficacy of those treatments.

The specific aims proposed to meet these goals are as follows.

Specific Aim 1. To induce p53-dependent inhibition of radiation- or chemotherapy-induced apoptosis by anti-apoptotic molecules.

Specific Aim 2. To develop a promoter specific for wild-type p53-expressing cells, using wild-type p53-DNA binding sites upstream from a minimal promoter.

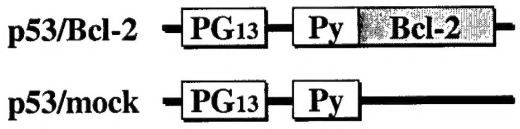
Specific Aim 3. To determine the extent to which anti-apoptotic molecules under the control of a novel p53-specific promoter (PGn) complexed with LPD can be used, under optimal conditions, to reduce the toxicity of radiation therapy or chemotherapy.

The goal for year 3 of the original application was to complete Specific Aim 3. Progress toward that goal is described in the remainder of this report.

Body

In year 1 and 2, we completed Specific Aim 1 and part of specific aim 2. In specific aim 2, we have shown that we can inhibit radiation- or chemotherapy-induced apoptosis by anti-apoptotic molecules Bcl-2 in p53 dependent manner in vitro (last year report). Further, we have constructed p53/Bcl-2 vectors, which contained the polyomavirus early promoter and bcl-2 gene located downstream of a DNA sequence (PG) that binds wild type (wt) p53 in vitro (Fig. 1a). To test whether wt p53 can induce Bcl-2 protein expression through this vector, we used H1299 lung cancer cells, which have homozygous deletion of the p53 gene. H1299 cells were co-transfected with p53/Bcl-2 and wt p53 vectors. 48 hours after transfection, wt p53 protein strongly upregulated Bcl-2 expression only in p53/Bcl-2 and wt p53 co-transfected cells.

(a)



(b)

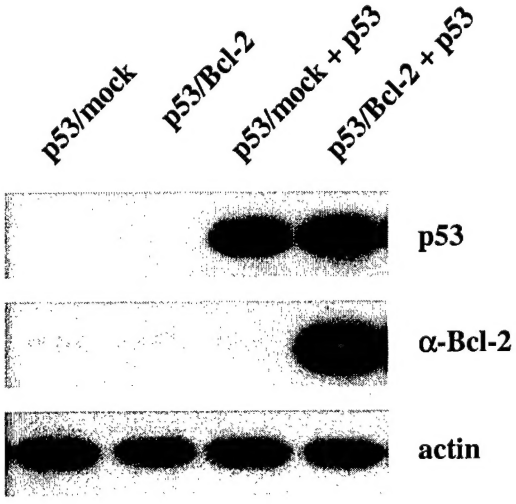


Fig. 1. p53/Bcl-2 vectors were activated by the wild type p53 protein in H1299 cells. (a) Construction of p53/Bcl-2 and p53/mock vectors. PG: the p53 binding sequence; Py: the early gene promoter from polyomavirus (b) H1299 cells were transfected with either p53/mock or p53/Bcl-2 vectors and co-transfected with wild type p53 vectors. Cells were harvested for Western blot analysis 48 hours after transfection and blots were probed for p53, Bcl-2 and actin. The expression of Bcl-2 protein was observed only in p53/Bcl-2 and p53 co-transfected cells. Actin was used as a loading control.

.Specific Aim 3 (Reduction of radiation- or chemotherapy-induced toxic effects in normal cells in mice bearing mutated p53 breast cancer xenografts) will be addressed in the third years of funding.

After we confirmed that p53/Bcl-2 can specifically express in wt p53 cells but not in cancer cells in vitro, we examined whether reduction of apoptosis can improve the outcome radiation induced toxicity. First, we carried out radiation dose–response studies used whole thorax irradiation model in mice to characterize the apoptotic response to radiation in the mice lung (Fig 2). Female NCR mice, 3-4 months old, were irradiated to the whole thorax irradiation doses of 10 to 30 Gy. The mice were sacrificed at 0, 6, and 24 hours after irradiation, and their lungs were removed. Fig. 2 shows the percentage of apoptotic cells in the lungs of mice after radiation treatment. The percentage of apoptotic bodies peaked at 6 h after irradiation. The incidence of spontaneous apoptosis was less than 0.1% in age-matched control mice. Apoptotic bodies in the lung were detected after irradiation a dose of 10 Gy and were maximal 20 Gy. Increasing the radiation dose to 30 Gy failed to increase the apoptotic response in the mice lung. Therefore, we chose the radiation dose of 20 Gy as following experiment.

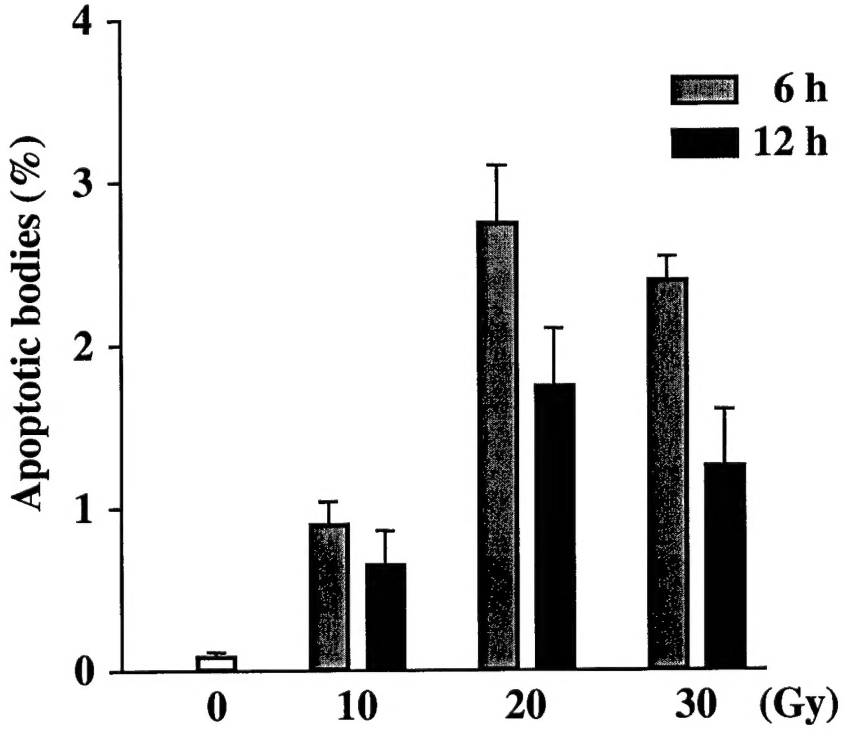


Fig. 2. **Induction of apoptosis in mice lung by radiation.** Female NCR/Sed-*nu*/+ (NCR)

mice, bred and maintained in a specific pathogen-free colony, were used. They were housed five to a cage and were given sterilized food and sterilized acidified water in an animal colony approved by the American Association for Accreditation of Laboratory Animal Care and in accordance with current regulations and standards of the United States Department of Agriculture and Department of Health and Human Services, National Institutes of Health. The mice were 2-3 months old at the time of irradiation. Groups of four unanesthetized mice were an irradiated simultaneously through a ventral portal using a Phillips 250-kVp x-ray unit at a setting of 250 kVp, 15 mA at 80% output, with a skin surface distance of 35.5 cm and a half-value layer of 0.5 mm Cu. Animals were restrained in a specially designed Lucite jig used routinely in this laboratory for lung irradiation. The whole thorax was irradiated through a 25 mm wide and 22 mm long portal on the ventral surface of the mouse. The remainder of the body was shielded with 3.2 mm of lead. The dose rate was 1.05 Gy/min.

Fig. 3 shows the percentage of apoptotic cells in the mice lung. All mice received 20 Gy whole lung irradiation with PG_{13}-mock, PG_{13}-Bcl-2, or CMV-Bcl-2 plasmids. Apoptotic bodies were reduced by more than 50 % after irradiation in the mice given the PG_{13}-Bcl-2 or CMV-Bcl-2 plasmids. The percentage of apoptosis was calculated based on total no. of cells in the microscopic field/ However, give that LPD cationic liposome predominantly transfect endothelial cells and recent report suggesting apoptosis in endothelial cells may be the major factor related to radiation toxicity. We are restaining the lung tissue with Factor VIII to distinguish endothelial cells from alveolar cells. This allows calculating the percentage of apoptotic cells based on total endothelial cells as denominator.

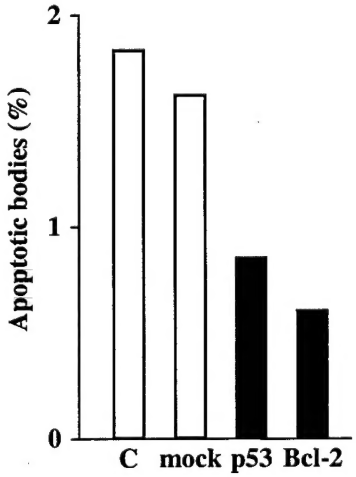


Fig. 3. **Induction of apoptosis in mice lung by radiation.** Twenty-four hours before irradiation of Female NCR/Sed-*nu*/+ (NCR) mice as described in figure 3, mice were injected via a tail vein with vehicle only (DOTAP-cholesterol/protamine sulfate liposome), LPD- PG_{13}-mock, LPD-PG_{13}-Bcl-2, or LPD-CMV-Bcl-2 (20 μg DNA).

Key Research Accomplishments

- **Bcl-2 can be specifically expressed in cells with wild type 53 using p53/Bcl-2 expression vector.**
- **Reduction of apoptosis of lung tissue was observed by both Bcl-2 and p53/Bcl-2 expression vector**

Reportable Outcomes

The findings described in here have been presented at AACR. We are currently preparing a manuscript that will be submitted to Cancer Research for protection from radiation-induced apoptosis specifically in wild type p-53 cells. We are also currently repeating some of the experiments in specific aim 3.

Conclusions

We have shown that ectopic overexpression of Bcl-2 counters the cytotoxicity of doxorubicin (Adriamycin), paclitaxel (Taxol), and radiation in the first year. In the second year, we showed that Bcl-2 expression can be induced specifically in cells with wild-type p53, in particular under genotoxic stress (irradiation) when cells were transfected with a heterogeneous promoter of a wild-type p53-specific promoter combined with Bcl-2 cDNA. These findings indicate that normal cells expressing wild-type p53, but not p53-mutated or p53-deleted breast cancer cells, can be protected from at least some of the effects of radiation by inducing the wt p53-specific expression of Bcl-2. In the third year that, newly generated Bcl-2 expression vector, which is dependent of p53, can reduce apoptosis in animals treated with radiation, which may reduce pulmonary toxicity.

References

N/A

Appendices

N/A